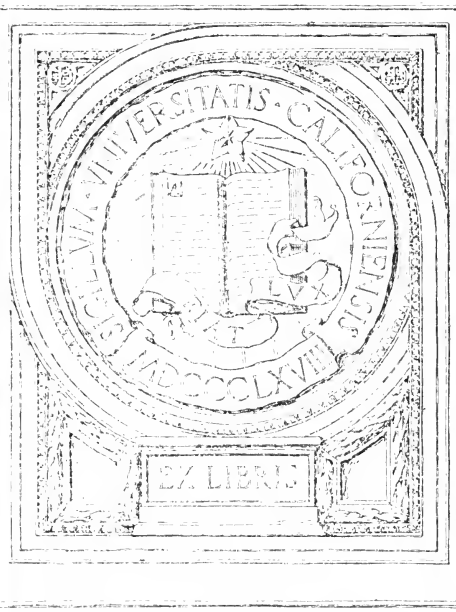
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# The Influence of Alpha—Methyl Glucoside on the Invertase Hydrolysis of Sucrose

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## DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE IN COLUMBIA UNIVERSITY

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BY

GUSTAVE E. LANDT, B.S.

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NEW YORK CITY

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To Winifred M.

DEDICATION

## ACKNOWLEDGMENTS

The author wishes to acknowledge his indebtedness to Professor John M. Nelson, who suggested and directed this investigation.

ABSTRACT of Dissertation

1. What was attempted.
2. The extent to which this attempt was successful.
3. The contributions actually new to the Science of Chemistry that have been made.

1. The presence of alpha-methyl glucoside in the invertase hydrolysis of sucrose has a very marked effect on the velocity of reaction. It was therefore thought desirable to study the effect of this substance on various factors in the hydrolysis, and to explain these effects if possible. Before this could be done, it was necessary to have more precise information on the unretarded hydrolysis of sucrose than has been available. Incidental, therefore, to the work on retardation, a study of the relation of sucrose concentrations and velocities of reaction was undertaken.

2. It has been found that:

(a) Up to a concentration of 4.5% sucrose, the initial velocities of reaction approximate a straight line function of the logarithm of the molar sucrose concentration. A rather sharp change then occurs and the initial velocities become independent of the sucrose concentrations within the ranges studied. These facts are irreconcilable with the theory of invertase action advanced by Michaelis and Menten.

(b) The position of the change in the relationship between sucrose concentrations and velocities appears to be independent of the concentration of invertase.

(c) It has also been shown that alpha-methyl glucoside causes the concentration where maximum velocity is attained, to be changed. This is the first instance where an effect of this character has been observed.

(d) A further study of the retarding effect of alpha-methyl glucoside indicates that one of the causes of its effect is compound formation between it and invertase.

3. The following contributions to the Science of Chemistry have been made.

(a) It has been shown that the concentration where maximum velocity is attained corresponds to a rather definite and narrow range of concentrations, (at about 4.5% sucrose).

(b) The value of this concentration is independent of the invertase concentration.

(c) The value of this concentration is changed by the presence of alpha-methyl glucoside.

(d) Evidence is obtained for compound formation between alpha-methyl glucoside and invertase.

# THE INFLUENCE OF ALPHA-METHYL GLUCOSIDE ON THE INVERTASE HYDROLYSIS OF SUCROSE.

Brown[1], (J. Cehm. Soc., 81; 373) and more recently Nelson and Vosburg[2] (J. A. C. S. 39; 790; 1917) found that in the hydrolysis of sucrose by invertase, the rate of reaction increased more and more slowly as the concentration of substrate was increased, and that finally it reached a maximum beyond which additional amounts of substrate had no influence on the velocity. The last named authors also pointed out that this maximum rate was obtained at a rather definite concentration of sucrose, ranging between 4% and 6%.

Michaelis and Menton[3], (Biochem. Zeit. 49; 333; 1913) by plotting the relative initial velocity of hydrolysis of sucrose by invertase against the logarithm of the initial molar sucrose concentration, obtained data which are represented by the points in Figure 1. (The relative rates are compared by considering the maximum rate as 100 and the other rates as percents of this maximum.) These points they conclude, indicate a "dissociation-rest curve" (discussed on page 20 represented by the heavy graph in the same figure. That is, they claim their results show that invertase and sucrose combine to form a compound according to the Mass Law and that the rate of hydrolysis is proportional to the amount of invertase-sucrose compound present. This view of the mechanism involved in the reaction has aroused considerable interest among investigators in the field of Enzyme Chemistry. Euler[4], (Chemie der Enzyme, Munich, 1920) considers it to be a contribution equal in importance to Sorensen's study of hydrogen ion concentrations as related to enzymes.

In attempting to determine the concentration of sucrose corresponding to the maximum velocity of hydrolysis, the present author adopted Michaelis and Menten's method of plotting the average initial velocity against the logarithm of the initial molar sucrose concentrations, instead of against the sucrose concentration directly as was done by Nelson and Vosburgh[5]. (loc. cit.).

The results obtained were practically identical with those of Michaelis and Menten, but the familiarity gained in the calculation and plotting of the results in this manner has led to the conclusion that the discrepancy between the experimental curve and the theoretical curve is of such magnitude as to throw considerable doubt on Michaelis and Menten's claim.

In Table 1 are given the results from three series of hydrolysis, series A, B and C run under the conditions indicated. In column 3 of this table are given the values of the average initial velocities for the corresponding sucrose concentrations. These values were obtained as described below. In column 4 are given the ratios of the individual rates to the maximum rate.

The method employed in following the change in the initial rate of inversion with change in the sucrose concentration was briefly as follows: separate solutions containing sucrose and invertase respectively, and also containing sufficient sodium citrate as buffer to furnish the final solution in which the hydrolysis took place with the required $P_h$, were kept in the thermostat for a considerable length of time before mixing. Samples were removed from the reacting solutions at definite time intervals, indicated in the tables, the hydrolyses were immediately stopped by means of sodium carbonate, and the samples were finally examined in the polariscope at 25°, using a 200 millimeter tube.

FIG. I

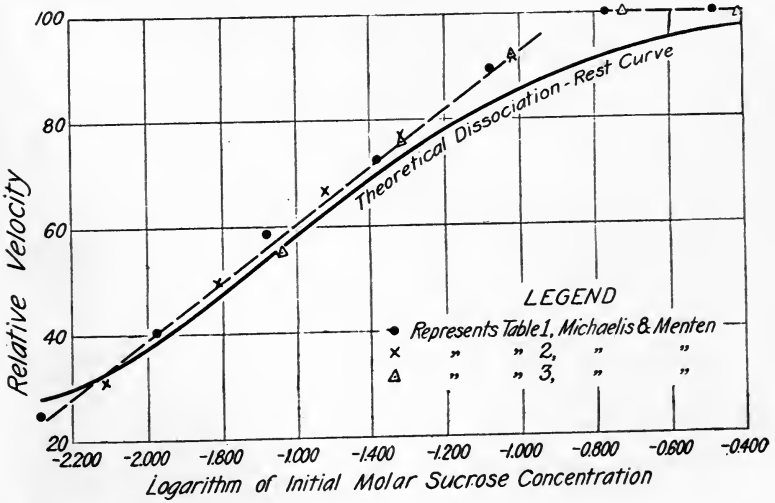


TABLE 1. SERIES A. CONDITIONS: $P_h = 4.6$; 3.06cc. invertase per 100cc. Temp. = 25°.

| Percent Conc. Suc. | Log. Mol. Conc. | Initial Velocity | Relative Velocity | Observed ration at time intervals in minutes. 0.0 | 1.0 | 2.50 | 5.0 | 7.5 | 10.0 | 15.0 | 20.0 | 30.0 | 40.0 | 50.0 | 60.0 | 90.0 | 150.0 | Inf. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.50 | —1.835 | 62.0 | .477 | 0.70 | 0.60 | 0.48 | 0.32 | 0.18 | 0.10 | .... | .... | .... | .... | .... | .... | .... | .... | —0.15 |
| 1.00 | —1.534 | 81.6 | .630 | 1.34 | .... | 1.00 | 0.73 | .... | 0.31 | 0.03 | —0.15 | .... | .... | .... | .... | .... | .... | —0.36 |
| 1.50 | 1.358 | 97.7 | .759 | 1.98 | .... | 1.57 | 1.24 | .... | 0.65 | 0.20 | —0.09 | .... | .... | .... | .... | .... | .... | —0.55 |
| 2.00 | —1.233 | 108.0 | .890 | 2.60 | .... | .... | 1.78 | .... | 1.08 | 0.58 | 0.24 | —0.18 | .... | .... | .... | .... | .... | —0.78 |
| 3.00 | —1.056 | 118.0 | .903 | 3.91 | .... | .... | 2.95 | .... | 2.11 | 1.38 | 0.79 | —0.14 | .... | .... | .... | .... | .... | —1.17 |
| 4.00 | —0.932 | 127.0 | .974 | 5.24 | .... | .... | 4.21 | .... | 3.27 | .... | 1.64 | 0.44 | .... | .... | .... | .... | .... | —1.53 |
| 4.25 | —0.906 | 127.7 | .985 | 5.57 | .... | .... | 4.51 | .... | 3.56 | .... | 1.90 | 0.52 | .... | .... | .... | .... | .... | —1.62 |
| 4.50 | —0.881 | 129.6 | 1.000 | 5.90 | .... | .... | 4.82 | .... | 3.84 | .... | 2.21 | 0.88 | .... | .... | .... | .... | .... | —1.70 |
| 5.00 | —0.835 | 130.0 | 1.000 | 6.57 | .... | .... | 5.50 | .... | 4.54 | .... | 2.74 | 1.32 | 0.21 | —0.55 | .... | .... | .... | —1.80 |
| 5.50 | —0.793 | 129.4 | 1.000 | 7.22 | .... | .... | 6.14 | .... | 5.16 | .... | 3.35 | 1.84 | 0.63 | —0.26 | .... | .... | .... | —2.09 |
| 6.00 | —0.755 | 129.0 | 1.000 | 7.88 | .... | .... | 6.81 | .... | 5.80 | .... | 3.95 | 2.34 | 1.02 | 0.03 | —0.71 | .... | .... | —2.25 |
| 7.00 | —0.688 | 129.6 | 1.000 | 9.17 | .... | .... | 8.10 | .... | 7.07 | .... | 5.17 | 3.37 | 2.03 | 0.85 | —0.11 | .... | .... | —2.72 |
| 10.00 | —0.534 | 130.3 | 1.000 | 13.10 | .... | .... | 12.05 | .... | 11.03 | .... | 9.08 | 7.28 | .... | .... | 2.71 | —0.30 | —2.80 | —3.82 |

SERIES B. $P_h = 4.6$; 1.53cc. invertase per 100cc., Temp. = 25°

| | | | | Observed rotation in tim intervals in minutes 0.0 | 2.50 | 5.0 | 7.50 | 10.0 | Inf. |
|---|---|---|---|---|---|---|---|---|---|
| 0.50 | —1.835 | 35.0 | .537 | 0.68 | 0.55 | 0.45 | 0.36 | 0.28 | —0.17 |
| 1.00 | —1.534 | 43.0 | .657 | 1.24 | 1.07 | 0.92 | 0.79 | 0.64 | —0.45 |
| 2.00 | —1.233 | 52.9 | .805 | 2.61 | 2.39 | 2.17 | 1.95 | 1.74 | —0.77 |
| 3.00 | —1.056 | 59.8 | .917 | 3.89 | 3.65 | 3.40 | 3.16 | 1.94 | —1.17 |
| 4.00 | —0.932 | 63.9 | .977 | 5.23 | 4.98 | 4.71 | 4.44 | 4.21 | —1.53 |
| 4.25 | —0.906 | 64.0 | .980 | 5.57 | 5.30 | 5.04 | 4.79 | 4.55 | —1.62 |
| 4.50 | —0.881 | 65.4 | 1.000 | 5.88 | 5.61 | 5.34 | 5.08 | 4.85 | —1.70 |
| 5.00 | —0.835 | 65.3 | 1.000 | 6.45 | 6.18 | 5.91 | 5.65 | 5.41 | —2.00 |
| 6.00 | —0.755 | 65.5 | 1.000 | 7.82 | 7.56 | 7.31 | 7.06 | 6.81 | —2.25 |

SERIES C. $P_h = 4.6$; 7.65cc. invertase per 100cc., Temp. = 25°

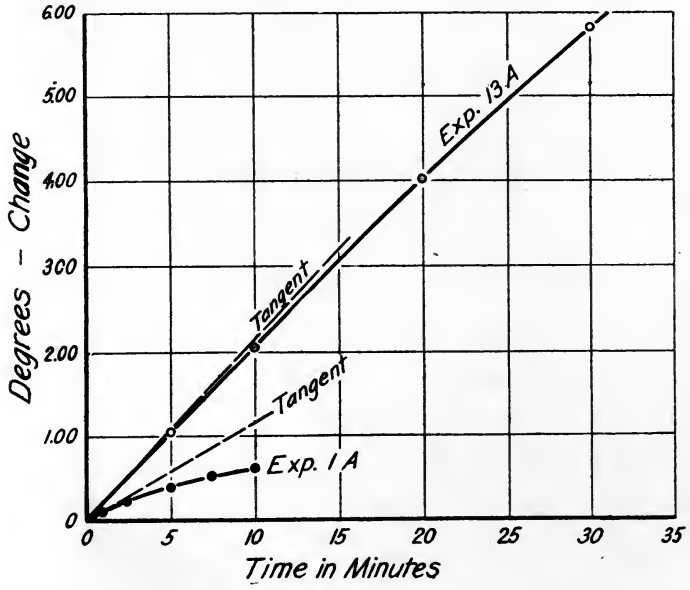
| | | | | Observed rotation in minute time intervals 0.0 | 1.0 | 2.0 | 3.0 | 4.0 | Inf. |
|---|---|---|---|---|---|---|---|---|---|
| 1.00 | —1.534 | 209.8 | .653 | 1.37 | 1.05 | 0.77 | 0.53 | 0.32 | —0.30 |
| 2.00 | —1.233 | 267.0 | .830 | 2.65 | 2.21 | 1.82 | 1.48 | 1.16 | —0.67 |
| 3.00 | —1.056 | 294.0 | .910 | 3.92 | 3.44 | 2.96 | 2.52 | 2.09 | —1.15 |
| 4.00 | —0.932 | 313.2 | .970 | 5.19 | 4.67 | 4.17 | 3.70 | 3.25 | —1.48 |
| 4.25 | —0.906 | 317.1 | .989 | 5.57 | 5.03 | 4.52 | 4.03 | 3.59 | —1.56 |
| 4.50 | —0.881 | 324.0 | 1.010 | 5.91 | 5.36 | 4.85 | 4.36 | 3.89 | —1.71 |
| 5.00 | —0.835 | 324.6 | 1.020 | 6.47 | 5.93 | 5.44 | 4.95 | 4.49 | —1.95 |
| 6.00 | —0.755 | 318.0 | .983 | 7.78 | 7.24 | 6.74 | 6.24 | 5.77 | —2.36 |

Velocities are given in milligrams of sucrose hydrolised per minute.

A quartz mercury lamp provided with a suitable light filter was used. (See Nelson and Hitchcock[1], (J. A. C. S. 43; 2632; 1921). The hydrogen ion concentration of the solution was determined by the electromotive force method, and also by means of indicators standardized against solutions whose known $P_h$ had been previously determined electrometrically. All hydrolyses were run in duplicate.

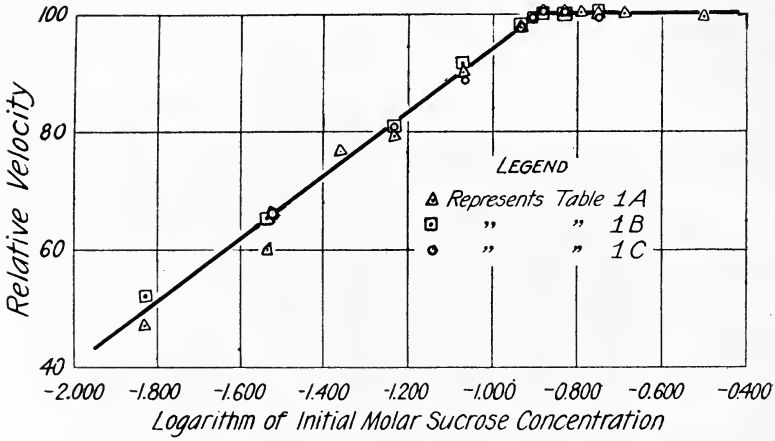
THE AVERAGE INITIAL VELOCITIES: By plotting the observed change in rotation against the time, T, as abcissa a curve relating the amount of sucrose hydrolysed and the time was obtained. The results from experiments 1 and 13 in Table 1A have been plotted in this manner in Figure 2 as an example. The values for the initial velocities were obtained by determining the value of the tangent to the reaction curve at the origin. The values of the tangents thus obtained were converted by means of the units employed for the coordinates, into milligrams of sucrose hydrolysed per minute. Four trial tangents were drawn by the inspection method, and their arithmetic mean was taken as the true value.

FIG. II



RELATIONSHIP OF VELOCITIES AND CONCENTRATIONS: The relative initial rates and the corresponding logarithms of the molar sucrose concentrations have been plotted in Figure 3, and it seems that a straight line up to the point of Maximum velocity more nearly fits the data than the curve of Michaelis and Menten in Figure 1. The dotted line drawn through their experimentally determined points also appears to approximate more closely a straight line than the dissociation-rest curve as they claim. This discrepancy between the dissociation-rest curve, Figure 1, and the experimentally determined points can not be ascribed to experimental error as the following discussion shows.

FIG. III




ESTIMATE OF ERROR: The precision of the results in Table 1A were determined, and from them the maximum deviation from the conclusion was estimated. As was mentioned, the average initial velocities are calculated in milligrams per minute from the value of the tangent drawn to the beginning of the reaction curves of the type shown in Figure 2. Since the initial parts of many of these curves were nearly straight, this could be done with considerable facility. The method of estimating the precision of the velocities was, first to determine the precision of the points in the reaction curves, and then to take into account how two factors cause the precision of the velocities, as calculated from the precision of the points to vary. These

factors are: the accuracy with which tangents can be read, and the time interval between taking samples from the reaction mixture. These specific influences will be discussed after we have determined the precision of the points on the reaction curves.

PRECISION OF THE POINTS ON THE REACTION CURVES: The errors in four operations contribute to the accuracy of the rotation of a reaction sample. These are, errors in reading the polariscope, in reading the time, and in making two measurements with the pipette. The average deviation of the polariscope readings was 0.01°. Since the mean of four readings was taken as the final measurement, the deviation measure of the arithmetic mean of the readings was .005°.

Samples of the reaction mixtures were pipetted into five cubic centimeters of sodium carbonate solution also measured with the pipette. The errors in pipetting were 0.01cc. Since these errors will change the concentrations of the reaction samples, the polariscope readings will be correspondingly in error. Their influence on the final precision is, however, only one-tenth that due to other errors. They are therefore not considered in the final estimate of the precision.

The error in reading the time of starting the reaction and of taking samples was estimated as one second. At and above the optimum, where we are chiefly concerned with the limits of error, this error in the time will cause an error of 0.005° in reading the polariscope.

Therefore, the precision of the observed rotation will be determined by the errors in reading the polariscope, and in timing the reaction samples, or an amount determined by the relation

$$\sqrt{0.005^{\circ 2} + 0.005^{\circ 2}} = \text{precision} = .007^{\circ}.$$

The points in the reaction curves were obtained by taking the difference between two readings. Therefore, the error in such a point may be twice that in each reading, or 0.014°. The precision of a point in the curve is therefore 0.014°.

ACCURACY IN READING TANGENTS: We will now discuss the factors which modify the precision of the initial velocities as determined from the above results. The first is the accuracy of reading the tangents. When the curve was almost straight, the tangent could be read with sufficient accuracy to discard the errors in reading them. This was true for the

curves for concentrations of 3% sucrose and greater. Thus at 3% the deviation of the mean in calculating the milligrams of sucrose hydrolysed per minute from errors in reading the tangents was 0.1 milligram. Below 3% sucrose, however, the errors in reading the tangents would have to be considered in the final estimate of the precision.

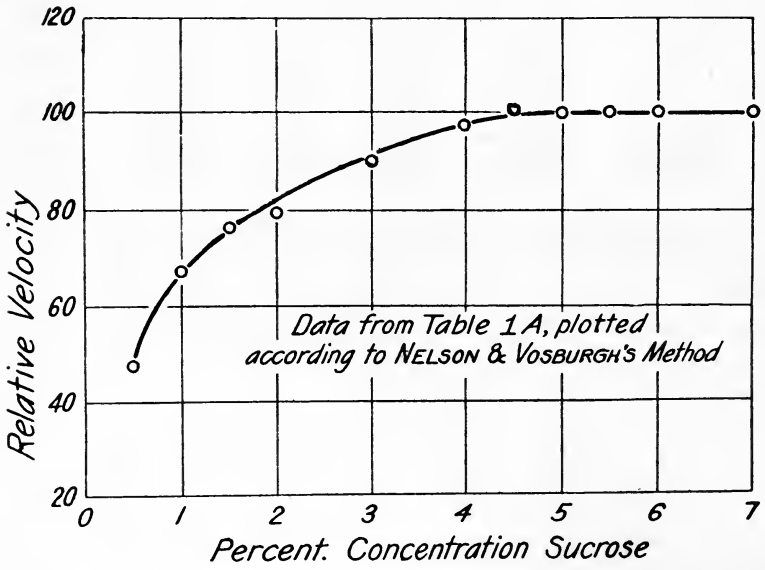
THE INFLUENCE OF TIME OF TAKING SAMPLES ON THE PRECISION OF THE VELOCITY: The initial velocity is the number of milligrams of sucrose hydrolysed in the first minute, if the reaction proceeds at the initial rate as indicated by the reaction curve. When the initial part of the curve is straight (or nearly so) and the first sample is read at one minute, then it is obvious that the initial velocity will be burdened by the total error of observation. If the first observation is made after five minutes, however, then the error in one minute (the first) will only be one-fifth the total error of observation. Thus the limits of error in the initial velocity are decreased when the time interval of taking samples is increased, providing the initial velocity is nearly constant. When the initial velocity is not nearly constant, samples have to be taken more frequently in order to establish the curvature. Thus the precision of the velocities will be lower at low concentrations where the initial velocity changes more rapidly, and where consequently samples must be taken more frequently. This conclusion is borne out by an inspection of the graph in Figure 3.

Since the possible error in one point on the reaction curve is .014°, and since at and above the concentration corresponding to maximum velocity, samples were taken at five minute intervals, the final estimate of the precision of the average initial velocities in this range is one-fifth this amount, or 2.5 milligrams when expressed in that unit. This error if plotted in Figure 3 would give a band two millimeters wide on each side of the curve around the optimum and above it. It can be seen, therefore, that this error cannot possibly account for the theoretical dissociation-rest curve plotted in Figure 1.

THE INFLUENCE OF CONCENTRATION OF INVERTASE: Nelson and Vosburgh[1] (loc. cit.) showed roughly that the sucrose concentration corresponding to the maximum rate of hydrolysis was independent of the amount of invertase employed. But due to plotting the relative rates against the

sucrose concentrations directly, they obtained a curve like that in Figure 4. This curve approaches the maximum rate so gradually that it is impossible to determine the concentration corresponding to the maximum velocity very closely. On the other hand, since practically an abrupt change in the shape of the curve at the point where the maximum rate is reached, see Figure 3, is obtained by the present method of plotting; that is the relative initial rates against the logarithm of the concentration, a much more exact value for the concentration of maximum velocity is obtained. In this case, the value is found to be between 4% and 5% sucrose. This more exact value also appears to be independent of the amount of invertase employed. Thus all the points indicated in Figure 3 and which correspond to the data given in Table 1, which was obtained from three series of hydrolyses with different concentrations of invertase, fall on the same graph.

FIG. IV.




THE EFFECT OF ALPHA METHYL GLUCOSIDE ON THE CONCENTRATION CORRESPONDING TO MAXIMUM VELOCITY: It is well known that alpha methyl glucoside exerts a marked retarding influence on the activity of invertase.

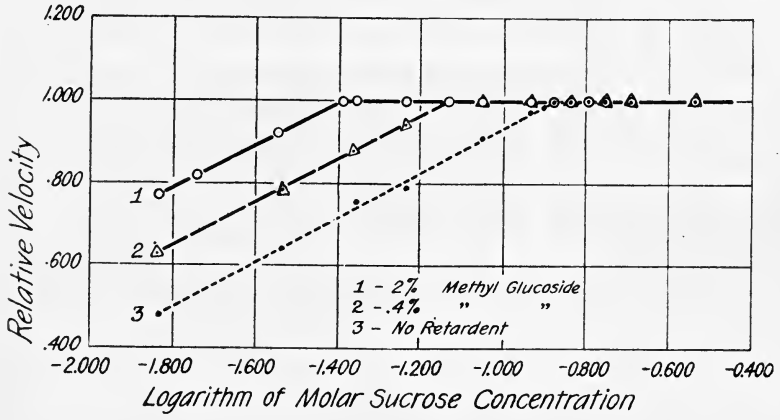
In the light of this, the effect of the retardant on the value of the sucrose concentration corresponding to maximum velocity has been examined. For this purpose, two series containing in the reacting solutions 0.4% and 2% alpha methyl glucoside respectively, were run similarly to those in which no alpha methyl glucoside was present. The method of procedure followed was the same, excepting that the glucoside was added to the sucrose solutions before the latter were mixed with the enzyme. The initial velocities obtained are given in Table 2 and are compared graphically in Figure 5 with the curve given in Figure 3, which, as was stated, corresponds to a series of hydrolyses of sucrose in the absence of alpha methyl glucoside. The experimental data from which the velocieties in Table 2 were calculated are given Experiments, Series D and E.

TABLE 2

| | | 0.4% Glucoside | | 2.0% Glucoside | |
|---|---|---|---|---|---|
| Percent. conc. sucrose | Log. Mol. conc. | Milligrams hyd. per min. | Relative velocity | Milligrams hyd. per min. | Relative velocity |
| 0.50 | —1.8350 | 28.0 | 0.630 | 11.38 | 0.769 |
| 0.65 | —1.7912 | ..... | ..... | 12.10 | 0.818 |
| 1.00 | —1.5340 | 34.2 | 0.763 | 13.60 | 0.920 |
| 1.40 | —1.3883 | ..... | ..... | 14.70 | 1.000 |
| 1.50 | —1.3579 | 40.5 | 0.910 | 14.82 | 1.000 |
| 2.00 | —1.2330 | 42.1 | 0.945 | 14.82 | 1.000 |
| 2.50 | —1.1361 | 44.45 | 1.000 | ..... | ..... |
| 3.00 | —1.0569 | 44.45 | 1.000 | 18.82 | 1.000 |
| 4.00 | —0.9319 | 44.90 | 1.000 | 14.90 | 1.000 |
| 5.00 | —0.8350 | 44.70 | 1.000 | 14.70 | 1.000 |
| 6.00 | —0.7558 | 44.35 | 1.000 | 14.68 | 1.000 |
| 7.00 | —0.6889 | 44.40 | 1.000 | ..... | ..... |
| 10.00 | —0.5340 | 44.00 | 1.000 | 14.70 | 1.000 |

The data for these was obtained from Experiments, Series D and E.

FIG. V



EXPERIMENTS: Series D. CONDITIONS: 0.4% alpha methyl glucoside; Temp. = 25°. $P_h$ = 4.6; 3.06cc. invertase per 100 cc. of solution; varying sucrose concentration.

| Percent | Rotation in degrees after—minutes. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. Suc. | 0.00 | 2.50 | 5.00 | 7.50 | 10.00 | 20.00 | 30.00 | 40.00 | 50.00 | 70.00 | 90.00 | 120.0 | 150.0 | 180.0 | Inf. |
| 0.50 | 1.91 | 1.80 | 1.70 | 1.62 | 1.54 | 1.26 | .... | .... | .... | .... | .... | .... | .... | .... | 1.06 |
| 1.00 | 2.56 | .... | 2.29 | .... | 2.02 | 1.53 | 1.25 | 1.09 | .... | .... | .... | .... | .... | .... | 0.90 |
| 1.50 | 3.22 | .... | 2.88 | .... | 2.54 | 1.97 | 1.44 | 1.11 | .... | .... | .... | .... | .... | .... | 0.73 |
| 2.00 | 3.87 | .... | 3.51 | .... | 3.17 | 2.46 | 1.90 | 1.39 | 1.01 | 0.65 | .... | .... | .... | .... | 0.53 |
| 2.50 | 4.55 | .... | 4.18 | .... | 3.83 | 3.13 | 2.46 | .... | 1.39 | 1.29 | .... | .... | .... | .... | 0.35 |
| 3.00 | 5.17 | .... | .... | .... | 4.43 | .... | 3.03 | .... | 1.83 | 0.93 | 0.44 | .... | .... | .... | 0.15 |
| 4.00 | 6.45 | .... | 6.08 | .... | 5.72 | 4.93 | 4.21 | .... | 2.84 | 1.65 | 0.76 | 0.03 | .... | .... | —0.23 |
| 5.00 | 7.75 | .... | 7.39 | .... | 6.99 | 6.25 | 5.50 | .... | 4.11 | 2.79 | 1.65 | 0.43 | —0.22 | .... | —0.61 |
| 6.00 | 9.08 | .... | 8.72 | .... | 8.38 | 7.58 | 6.82 | .... | 5.40 | 4.03 | 2.75 | 1.15 | 0.09 | .... | —1.02 |
| 7.00 | 10.32 | .... | 9.94 | .... | 9.57 | 8.82 | 8.07 | .... | 6.58 | 5.16 | 3.82 | 2.04 | 0.63 | —0.33 | —1.39 |
| 10.00 | 14.24 | .... | 13.89 | .... | 13.51 | 12.79 | 12.04 | .... | 10.52 | 9.05 | 7.59 | 5.52 | 3.62 | 1.93 | —2.56 |



EXPERIMENTS: Series E. CONDITIONS: Same as above excepting a concentration of 2% alpha methyl glucoside.

| Percent | Rotation in degrees after—minutes. | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. Suc. | 0.00 | 5.00 | 10.00 | 20.00 | 30.00 | 60.00 | 90.00 | 120.0 | 150.00 | 180.00 | 240.0 | 300.0 | 360.0 | 420.0 | 540.0 | 600.0 | Inf. |
| 0.50 | 6.81 | 6.71 | 6.61 | 6.45 | 6.29 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.92 |
| 0.65 | 6.98 | 6.88 | 6.77 | 6.59 | 6.39 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.86 |
| 1.00 | 7.49 | 7.38 | 7.27 | .... | 6.85 | 6.32 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.82 |
| 1.40 | 8.01 | .... | 7.77 | 7.52 | 7.30 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.68 |
| 1.50 | 8.18 | .... | 7.94 | 7.69 | 7.46 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.64 |
| 2.00 | 8.78 | 8.66 | 8.54 | .... | 8.07 | 7.42 | 6.82 | 6.24 | 5.85 | .... | .... | .... | .... | .... | .... | .... | 5.47 |
| 3.00 | 10.07 | 9.94 | 9.83 | .... | .... | 8.69 | .... | 7.39 | .... | 6.24 | .... | .... | .... | .... | .... | .... | 5.06 |
| 4.00 | 11.37 | 11.25 | .... | .... | 10.62 | 9.91 | 9.24 | 8.57 | 7.88 | 7.33 | 6.15 | .... | .... | .... | .... | .... | 4.70 |
| 5.00 | 12.57 | .... | .... | .... | 11.84 | 11.14 | 10.34 | 9.72 | 9.00 | 8.40 | 7.11 | 6.02 | .... | .... | .... | .... | 4.31 |
| 6.00 | 13.85 | .... | .... | .... | 13.13 | 12.41 | .... | 10.97 | .... | 9.58 | 8.28 | 7.07 | 5.87 | 4.98 | .... | .... | 3.77 |
| 7.00 | 15.06 | .... | .... | .... | 14.34 | 13.59 | .... | 12.17 | .... | 10.79 | 9.44 | 8.16 | 6.86 | 5.75 | 4.04 | .... | 3.50 |
| 10.00 | 19.07 | .... | .... | .... | .... | 17.62 | .... | .... | .... | 14.69 | .... | 11.87 | 10.49 | 9.14 | 6.68 | 5.52 | 2.44 |

Graphs 1 and 2 in Figure 5 show 1.4% and 2.5% sucrose respectively to be the sucrose concentrations at which the maximum velocities are reached. These values are different from 4.5% obtained when no glucoside is present. In other words, the presence of the glucoside changes the concentration of maximum velocity.

In attempting to account for this effect of the alpha methyl glucoside, it was decided to examine the retarding influence of varying amounts of glucoside upon the rate of reaction by means of a series of solutions, all containing the same concentration of sucrose and invertase. The procedure followed was the same as before. The results obtained are tabulated in Experiments, Series F. The velocities calculated from these results are given in Table 3, and are represented graphically in Figure 6.

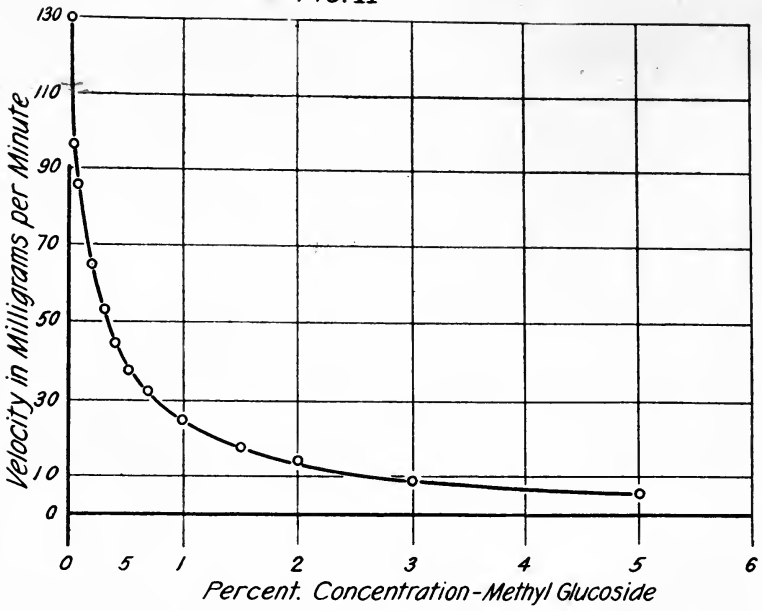
TABLE 3

| Percent conc. glucoside | Log. Mol. concentration | Initial Velocity | Milligrams retarded per min. | R | K |
|---|---|---|---|---|---|
| 0.000 | 0.000 | 129.6 | 0.0 | 0.000 | ..... |
| 0.025 | —2.8899 | 105.5 | 24.5 | 0.186 | .00549 |
| 0.05 | —2.5888 | 97.3 | 32.3 | 0.249 | .00878 |
| 0.10 | —2.2878 | 86.2 | 43.4 | 0.335 | .01024 |
| 0.20 | —1.0868 | 65.0 | 65.0 | 0.500 | .01030 |
| 0.30 | —1.8107 | 54.3 | 75.3 | 0.581 | .01115 |
| 0.40 | —1.6857 | 44.9 | 84.7 | 0.654 | .01091 |
| 0.50 | —1.5888 | 39.2 | 90.4 | 0.697 | .01119 |
| 0.70 | —1.4427 | 34.4 | 95.2 | 0.734 | .01308 |
| 1.00 | —1.2878 | 24.7 | 104.9 | 0.809 | .01224 |
| 1.50 | —1.1117 | 17.9 | 111.6 | 0.861 | .0125 |
| 2.00 | —0.9868 | 14.4 | 115.2 | 0.900 | .0111 |
| 3.00 | —0.8107 | 9.6 | 120.0 | 0.926 | .0112 |
| 5.00 | —0.5888 | 7.2 | 122.4 | 0.9444 | .0150 |
| 7.00 | —0.4427 | 4.8 | 124.7 | 0.962 | .0114 |

It becomes evident from the shape of the graph in Figure 6 that the retarding influence is not directly proportional to the concentration of the glucoside. As the concentration of the glucoside is increased, the retarding influence increases more and more slowly. As a result, the curve in Figure 6 appears to be asymptotic to the axis of abscissas.

The retardation resulting from a definite amount of glucoside is the difference in velocity between unretarded and retarded reactions of the same sucrose concentrations. Since the graph in Figure 6 approaches the axis of abscissas asymptotically it was assumed that maximum retardation will be complete cessation of the reaction. This maximum retardation was designated

FIG. VI




as 100 and the retardation at the other concentrations of glucoside as percents of this maximum. These values are also given in Table 3 in the column headed R′, and are plotted against the logarithms of the initial molar glucoside concentrations in Figure 7. Consideration of the following discussion will show that this method of plotting data gives a dissociation-rest curve on the basis of certain assumptions that are laid down therein.

Michaelis and Menten[1] (loc. cit.) obtain their dissociation-rest curves by relating the concentrations of undissociated residue (AB) and the concentration of one of the components (A) as they occur in the general dissociation reaction

$$AB = A + B$$

as follows: R, the ratio of (AB) to the maximum amount of (AB) formed when dissociation is completely suppressed, is designated by the expression

$$R = \frac{(AB)\ \text{obs.}}{(AB)\ \text{max.}}$$

then from the Law of Mass action it follows that

EXPERIMENTS: Series F. CONDITIONS: 10% sucrose; $P_h$ = 4.06; Temp. = 25°; 3.06cc. invertase per 100cc. of solution; varying amounts of alpha methyl glucoside.

| Percent Conc. of Glucoside | Rotation after the reaction has proceeded for—minutes. 0.00 | 4.00 | 8.00 | 15.0 | 30.0 | 45.0 | 60.0 | 90.0 | 120 0 | 150.0 | 180.0 | 210.0 | 240.0 | 300.0 | 360.0 | 420.00 | 480.0 | 540.0 | 600.0 | Inf. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00 | See Table 1A, preceeding part of paper. | | | | | | | | | | | | | | | | | | | |
| 0.025 | 13.18 | 12.48 | 11.78 | 10.56 | 8.06 | 5.83 | 3.83 | 0.74 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | —3.80 |
| 0.050 | 13.26 | 12.59 | 11.95 | 10.82 | 8.51 | 6.34 | 4.39 | 12.6 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | —3.70 |
| 0.100 | 13.41 | 12.81 | 12.20 | 11.22 | 9.13 | 7.19 | 5.33 | 2.17 | —0 17 | .... | —2.47 | .... | .... | .... | .... | .... | .... | .... | .... | —3.43 |
| 0.200 | 13.61 | 13.18 | 12.65 | 11.38 | 10.17 | .... | 6.93 | .... | . .. | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | —3.25 |
| 0.300 | 13.94 | .... | 13.22 | 12.60 | 11.22 | .... | 8.53 | 5.97 | 3 64 | 1.66 | 0.08 | .... | .... | .... | .... | .... | .... | .... | .... | —2.86 |
| 0.400 | 14.24 | See Experiments series D for data. | | | | | | | | | 1.93 | 0.57 | .... | .... | .... | .... | .... | .... | .... | —2.56 |
| 0.500 | 14.54 | 14.31 | 14.05 | 13.54 | 12.54 | .... | 10.54 | 8.53 | 6 60 | .... | 3.16 | 1.71 | 0.46 | —1.51 | .... | .... | .... | .... | .... | —2.25 |
| 0.700 | 15.16 | .... | .... | 14.28 | 13.44 | .... | 11.82 | 10.21 | 8 62 | 7.18 | 5.70 | .... | 3.01 | 0.87 | .... | .... | .... | .... | .... | —1.43 |
| 1.000 | 16.06 | .... | .... | .... | 14.81 | .... | 13.51 | .... | 11 04 | 9.79 | 8.57 | 7.39 | .... | 4.08 | 2.27 | .... | 0.39 | .... | .... | —0.70 |
| 1.500 | 17.60 | .... | .... | .... | 16.71 | .... | .... | 14.83 | . .. | .... | .... | 11.23 | .... | 8.60 | 6.94 | 5.38 | 3.99 | .... | .... | 0.82 |
| 2.000 | 19.07 | .... | .... | .... | .... | .... | 17.64 | .... | . .. | .... | 14.69 | .... | .... | 11.87 | 10.49 | 9.14 | 7.86 | 6.68 | 5.52 | 2.44 |
| 3.000 | 22.25 | .... | 22.12 | .... | 21.77 | .... | 21.29 | .... | 20.33 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 5.65 |
| 5.000 | 28.31 | .... | 28.21 | .... | 27.95 | .... | 27.59 | .... | 26.87 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 11.81 |
| 7.000 | 34.49 | .... | 34.42 | .... | 34.25 | .... | 34.00 | .... | 33.52 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 18.09 |

EXPERIMENTS: Series G. CONDITIONS: 5% sucrose; $P_h$ = 4.6; Temp. = 25°; 3.06cc. invertase per 100cc. of solution; varying amounts of alpha methyl glucoside.

| Percent Conc. of Glucoside | Rotation after the reaction has proceeded—minutes. 0.00 | 5.0 | 10.0 | 20.0 | 30.0 | 60.0 | 75.0 | 90.0 | 120.0 | 150.0 | 180.0 | 240.0 | 300.0 | Inf. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 6.57 | 5.50 | 4.54 | 2.74 | 1.32 | —1.03 | .... | .... | . .. | .... | .... | .... | .... | —1.90 |
| 0.025 | 6.66 | 5.78 | 4.90 | 3.20 | 1.60 | .... | .... | .... | . .. | .... | .... | .... | .... | —1.79 |
| 0.100 | 6.81 | .... | 5.36 | 4.04 | 2.84 | 0.10 | —0.66 | .... | . .. | .... | .... | .... | .... | —1.64 |
| 0.400 | 7.75 | 7.39 | 6.99 | 6.25 | 5.50 | .... | 2.54 | 1.65 | 0.43 | —0.30 | .... | .... | .... | —0.61 |
| 1.000 | 9.53 | .... | .... | .... | 8.33 | 7.12 | .... | 5.95 | 4.84 | 3.81 | .... | .... | .... | 1.15 |
| 2.00 | 12.57 | .... | .... | .... | 11.84 | 11.14 | 10.78 | 10.34 | 9.72 | 9.00 | 8.40 | 7.18 | 6.02 | 4.31 |
| 3.000 | 15.72 | .... | 15.56 | 15.40 | 15.23 | 14.74 | .... | .... | .... | .... | .... | .... | .... | 7.42 |
| 5.000 | 21.78 | .... | 21.66 | 21.55 | 21.41 | 21.06 | .... | .... | . .. | .... | .... | .... | .... | 13.50 |
| 7.000 | 27.96 | .... | 27.88 | 27.79 | 27.71 | 27.47 | .... | .... | .... | .... | .... | .... | .... | 19.76 |

| | Conditions: Same as above only 2% sucrose | | | | | | | | Inf. |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 2.60 | 1.78 | 1.08 | 0.24 | —0.18 | (Time) | .... | .... | —0.78 |
| 0.10 | 2.89 | 2.25 | 1.67 | 0.77 | 0.17 | ( 50 ) | .... | .... | —0.43 |
| 0.400 | 3.87 | 3.51 | 3.17 | 2.50 | 1.90 | 1.01 | .... | .... | 0.53 |
| 1.000 | 5.70 | 5.52 | 5.35 | 4.94 | 4.60 | .... | 3.58 | .... | 2.35 |
| 2.000 | 8.78 | 8.66 | 8.53 | .... | .... | .... | 7.47 | 6.82 | 6.39 | 5.46 |
| 3.000 | 11.75 | .... | 11.59 | 11.43 | 11.27 | 11.00 | .... | .... | .... | 8.47 |
| 5.000 | 17.81 | .... | 17.69 | 17.57 | 17.44 | 17.26 | .... | .... | .... | 14.48 |
| 7.000 | 23.99 | .... | 23.91 | 23.82 | 23.74 | 23.66 | .... | .... | .... | 20.62 |

$$R = \frac{(A)}{(A) + k}$$

where k is the equilibrium constant. R when plotted against the logarithm of (A) gives a curve *whose shape is the same* for all reactions of the above type, and whose midpoint is determined by the value of k. Curves of this type are called "dissociation-rest curves" by Michaelis.*

If the glucoside is assumed to combine with the invertase according to the following equation,

Invertase + Glucoside = (Invertase-glucoside comp.)

then the application of the Mass Law as above gives,

$$(2) \qquad R' = \frac{(\text{Glucoside})}{(\text{Glucoside}) + k}$$

Furthermore, if the invertase combined with the glucoside is assumed to be inactive, then the retardation will be proportional to the concentration of the invertase-glucoside compound. Then equation 2 can be written

$$(3) \qquad \frac{\text{Retardation}}{\text{max. Retardation}} = \frac{(\text{Glucoside})}{(\text{Glucoside}) + k} = R'$$

In Figure 7, the values of the R′ obtained from Table 3 have been plotted against the logarithm of the molar glucoside concentration, using the concentration of added glucoside on the assumption that the amount removed to form the compound is negligibly small. The graph thus obtained has the shape of a dissociation-rest curve like the heavy line in Figure 1. That it is a dissociation-rest curve is also confirmed by the fact that when the above values for R′ and the concentration of glucoside are substituted in equation 3, constant values for k (see last column, Table 3) are obtained except for the extreme lower concentrations of the glucoside, (Curve 2 in Figure 7 has the characteristic

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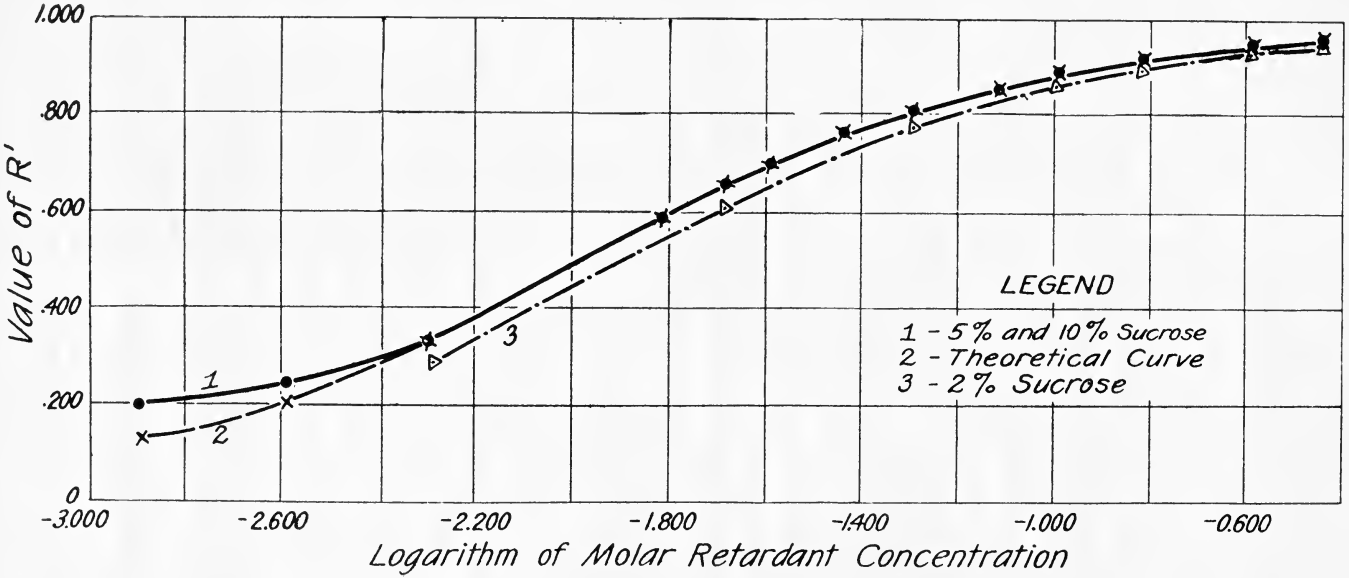
*Michaelis and Menten assume compound formation between invertase and sucrose according to the equation,

$$I + S = IS$$

and derive an analogous expression relating sucrose concentrations and the concentrations of the compound formed. The values of R are then the ratio of the individual velocities to the maximum on the assumption that the hydrolysis is due to the dissociation of the above compound into invert sugar and invertase. The rate of reaction would then be proportional to the concentration of the above compound.

FIG. VII


1.000
.800
.600
.400
.200
0
Value of R'
-3.000
-2.600
-2.200
-1.800
-1.400
-1.000
-0.600
Logarithm of Molar Retardant Concentration
1
2
3
LEGEND
1 - 5% and 10% Sucrose
2 - Theoretical Curve
3 - 2% Sucrose

shape of the dissociation-rest curve and indicates graphically the coincidence in the shapes of the curves.) This, therefore, indicates that the assumptions laid down above are correct, and that the retardation is due to inactivation of the invertase because of compound formation with the glucoside.

In order to see whether the concentration of sucrose might have an influence on this reaction, between the invertase and the glucoside, two additional series of experiments were run in which 5% and 2% sucrose solutions were hydrolysed in the presence of varying amounts of glucoside. The results obtained are given in Experiments, Series G, and the velocities and retardations calculated from these results are given in Table 4. The graph corresponding to the series in which the 5% sucrose solutions were used, coincides with that of the 10% solutions in Figure 7. In the case of the 2% sucrose solutions, however, this appears not to be the case, since the graph (dotted line, Figure 7) corresponding to this series falls slightly to the right of the graph for the 5% and 10% solutions. This abnormality is to be expected because the 2% sucrose solution is below the concentration which gives the maximum velocity, where according to Figures 1 and 3 the velocity is a linear function of the logarithm of the molar sucrose concentration. Because of this the results for this series would not be strictly comparable with those of the 5% and 10% series.

TABLE 4

| Percent conc. Glucoside | Log. Mol. conc. | Initial Velocity | Milligrams Retarded | R' |
|---|---|---|---|---|
| Sucrose conc. = 5% | | | | |
| 0.000 | 0.0000 | 129.6 | 0.0 | 0.000 |
| 0.025 | —2.8899 | 106.2 | 23.4 | 0.180 |
| 0.100 | —2.2878 | 87.3 | 42.3 | 0.326 |
| 0.400 | —1.6857 | 44.7 | 84.9 | 0.655 |
| 1.000 | —1.2878 | 24.1 | 105.5 | 0.813 |
| 2.000 | —0.9868 | 14.8 | 114.8 | 0.886 |
| 3.000 | —0.8107 | 9.6 | 120.0 | 0.926 |
| 5.000 | —0.5888 | 7.2 | 122.4 | 0.944 |
| 7.000 | —0.4427 | 4.9 | 124.7 | 0.966 |
| Sucrose conc. = 2% | | | | |
| 0.00 | 0.0000 | 108.8 | 0.0 | 0.000 |
| 0.10 | —2.2878 | 76.9 | 31.1 | 0.288 |
| 0.40 | —1.6857 | 42.1 | 65.9 | 0.610 |
| 1.00 | —1.2878 | 23.3 | 84.7 | 0.785 |
| 2.00 | —0.9868 | 14.6 | 93.4 | 0.865 |
| 3.00 | —0.8107 | 9.5 | 98.5 | 0.911 |
| 5.00 | —0.5888 | 7.1 | 100.9 | 0.933 |
| 7.00 | —0.4427 | 4.7 | 103.3 | 0.952 |

Velocities and retardations are given in milligrams per minute.

The action of alpha methyl glucoside cannot be entirely ascribed to compound formation, however, as consideration of Figures 3 and 5 shows, for in Figure 3 the concentration corresponding to maximum velociety is not changed by the addition of varying amounts of invertase, whereas in Figure 5 this concentration is changed. Thus other factors than compound formation must enter since this phenomenon cannot be ascribed to inactivation of the invertase by combination with the glucoside.

MATERIALS: The invertase used was a preparation made by Nelson and Hitchcock[1] (loc. cit.) and designated by them, as invertase number 8. This preparation did not change during the investigation as the following hydrolyses carried out at the beginning and end of the investigation show.

Hydrolysis of 10% sucrose solutions at 25° and $P_H$ =4.6 in presence of 3.06cc. invertase per 100cc of solution.

| Time | 0.00 | 5.00 | 10.00 | 30.00 | 60.00 | 90.00 | 180.0 | If. |
|---|---|---|---|---|---|---|---|---|
| Rotation | 13.10 | 12.05 | 11.03 | 7.28 | 2.71 | —0.30 | —2.80 | —3.82 |
| Rotation | 13.11 | 12.05 | 11.02 | 7.27 | 2.70 | —0.29 | —2.80 | —3.81 |

Two lots of sucrose were used. The one was prepared by the method of Bates and Jackson[2] (Bureau of Standards, Sci. Paper No. 268), from pure crystalized domino sugar, the second was obtained from the Pfannenstiehl Chemical Company. The rotation of these preparations agreed with that calculated from the theoretical equation of Landolt and Schoenock[3] (Landolt and Bornstein, Tabellen).

Secondary Sodium Citrate buffers were prepared according to the method of Clark[4] (The Determination of Hydrogen Ions, Williams and Wilkins, 1922.) C. P. chemicals were used thruout.

Alpha Methyl Glucoside was prepared according to the method of Fisher[5] (Berichte, 26; 2400.) and was purified by repeated crystalization from 95% alcohol. The highest specific rotation obtainable in this way was $159.26^5$ for the D line at 20° centigrade. This, therefore, is a better value for the specific rotation than that given by Fisher, as he obtained only 157.6°.

The ratio of the rotations of alpha methyl glucoside for the light of the D line and that of wave length 5416 u u was found to be 0.8486.

Alpha methyl glucoside was tested to see if invertase or hydrogen ion would hydrolyse it or cause it to muta-rotate. 0.5N HCl and 40cc. of invertase per 100cc. of solution used separately had no effect on its rotation in the course of a week.

The total change in rotation of sucrose when hydrolysed in the presence of alpha methyl glucoside was found to be less than its normal value, even after several days elapsed before the final reading of a reaction was made. That this was due to incomplete inversion of the sugar was shown to be the case by catalysing the hydrolysis of sugar with hydrogen ion in the presence of this retardant, in which case the change in rotation was the normal value. This was further established by allowing weeks to elapse before taking the final readings, in which case the usual change in rotation was obtained.

## SUMMARY

In the foregoing work, the relationship between reaction velocities and sucrose concentrations, when the initial velocities and initial concentrations were compared, has been investigated. The conclusions deduced from the experimental data are not in accord with the theory for invertase action advanced by Michaelis and Menten since the reaction velocities reach a maximum at a quite definite concentration of sucrose, rather than approach this maximum asymptotically as would be demanded by the theory of the above authors.

The effect of alpha methyl glucoside on the sucrose concentration where maximum velocity is attained has been studied. It has been shown to decrease this concentration very markedly. The significance of this effect lies in the fact that no other means of effecting a change in this concentration has yet been found.

An explanation of the retarding effect of alpha methyl glucoside was sought on the basis of certain assumptions which are based on the concept of compound formation between the invertase and the retardant. According to the assumptions used, a so-called dissociation-rest curve should be obtained. The experimental results coincide with the dissociation-rest curve so closely that the logical conclusion to be drawn is that the alpha methyl glucoside combines with the invertase and this compound is no longer capable of hydrolysing the sucrose.

## VITA

Gustave Landt was born in Highwood, Illinois, on November 25, 1894. He prepared for college at Tilden Technical High School in Chicago, Illinois, and entered the University of Chicago in 1914. He was graduated with honors in Chemistry and received the Degree of Bachelor of Science from that institution in March, 1918. From then till the end of the war he was engaged in chemical research in the service of the Chemical Warfare Service, U. S. A. The results of this work were embodied in several departmental reports and comprise investigations on Boron and Arsenic organic derivatives, and the recovery of Arsenious Oxide from Arsenic Acid. After demobilization in 1918 until September, 1919, when he entered Columbia University, he was in the employ of the Grasselli Chemical Company, engaged in research on the methods of manufacture of certain dyes and intermediates. Since 1919 he has been a graduate student under the Faculty of Pure Science at Columbia University. He has been an assistant in the Department of Chemistry since he entered and an instructor in University Extension since 1920. He is a member of the Phi Lambda Upsilon and Sigma Xi fraternities.